Brain, Behavior and Evolution
Editor-in-Chief: W. Riss, Brooklyn, N.Y.

Reprint
Publisher: S. Karger AG, Basel
Printed in Switzerland

Brain Behav. Evol. *23*: 14–25 (1983)

Motion-Induced Alterations in 2-Deoxyglucose Uptake in Brainstem Nuclei of Squirrel Monkeys: Autoradiographic and Liquid Scintillation Studies[1]

K.R. Brizzee, W.P. Dunlap

Delta Regional Primate Research Center, Covington, and Department of Psychology, Tulane University, New Orleans, La., USA

Key Words. Squirrel monkey · 2-Deoxyglucose · Double label · Motion sickness · Micropunch · Vestibular system · Liquid scintillation counting

Abstract. Each of 8 young adult female squirrel monkeys were injected via a femoral vein cannula with 167 μCi/100 g body weight of 2-(1,2-^{3}H)-deoxy-*D*-glucose (2-DG) (New England Nuclear, 37.3 Ci/mmol) in 0.5 ml sterile saline. 4 additional female squirrel monkeys were injected in the same manner with 100 μCi/100 g body weight of the (^{3}H)-2-DG. 2 h after this initial injection the original 8 animals were injected with 16.7 μCi/100 g body weight of 2-(1-^{14}C)-deoxy-*D*-glucose (51.3 mCi/mmol) in 0.5 ml sterile saline. The 4 additional animals were injected with 25 μCi/100 g body weight of the (^{14}C)-2-DG. Half of the animals at each dose level were restrained in the upright position with Velcro straps and a nontraumatic moulded plastic head holder on a modified animal restraint board [*Withrow and Devine*, 1972] with the head in the sagittal plane but tilted forward about 20°. They were then subjected to horizontal rotary motion at 25 rpm together with a vertical movement of 6 inches at 0.5 Hz for 1 h in a lighted room. The other half of the animals at each dose were restrained in the same manner and maintained in a quiescent state. At the end of this period each animal was anesthetized with ketamine, and the brain was quickly dissected out and frozen in isopentane cooled to −60 °C with dry ice. Transverse cryostat sections (−15 °C) of the brainstem were cut alternately at 200 and 20 μm from the nucleus gracilis caudally through the superior vestibular nucleus rostrally. Micropunch samples of the individual vestibular nuclei, and other brainstem nuclei and areas were obtained from the 200-μm sections with a modifi-

[1] This work was supported by NASA/Ames Research Center Grant NAG- 2-101 and NIH RR00164 and was conducted at Delta Regional Primate Research Center, Covington, La.

cation of the method of *Eik-Nes and Brizzee* with a small stainless steel punch measuring 850 μm in diameter. The frozen punch samples were prepared for liquid scintillation counting (Beckman LS7500 system). Differential (^{3}H) and (^{14}C) counts (cpm) were made employing external standards. The 20-μm sections were prepared for ^{14}C autoradiography by standard methods employing Kodak SB-5 X-ray film. The (^{14}C) autoradiographs from the motion-stimulated animals revealed a selectively high uptake of 2-DG in all of the vestibular nuclei, the lateral cuneate nucleus and the lateral reticular nucleus as compared with the corresponding structures in the control animals. The area postrema and nucleus of the solitary tract also exhibited somewhat higher grain density in motion-stimulated as compared with control subjects. The inferior olive showed a heavy 2-DG uptake in nearly all subjects but the brain density appeared somewhat higher in motion-stimulated animals. Data from the liquid scintillation counts revealed that the $^{14}C/^{3}H$ ratio (cpm) was significantly higher ($p < 0.05$) in the medial and inferior vestibular nuclei and nucleus cuneatus in motion-stimulated than in control animals. Values for the other vestibular nuclei and dorsal reticular formation (region of the vomiting center), and nucleus of the solitary tract were high but did not attain the level of statistical significance.

Introduction

In any discourse on the neurological basis of the emetic response to motion sickness it is evident that a consideration of the nerve pathways and nerve centers involved is a *sine qua non* to an ultimate understanding of the transmitter chemistry and physiological processes constituting the response. Since the nerve pathways are being discussed at some length by other authors in the Workshop, I will confine my remarks to the problem of the identification of functional centers involved in the response.

Studies on the sites of mediation of the vomiting reflex to various emetic stimuli date back to the investigations of *Thumas* in 1891. This worker destroyed an area in the caudal medulla oblongata in decerebrate dogs and found that animals with such lesions failed to exhibit the vomiting reflex in response to parenteral administration of apomorphine. In earlier studies he had noted that topical application of apomorphine to this area in decerebrate dogs consistently elicited emesis. He concluded that the vomiting center (VC) was located in deeper structures of a midline area, 2 mm wide and 5 mm long, extending through the obex.

Hatcher and Weiss [1922, 1923] confirmed *Thumas'* results with apomorphine in acute preparations but were able to elicit vomiting with orally administered mercuric chloride in dogs which were refractory to apomorphine. They destroyed the ala cinerea and reported that the animals were refractory to both intravenous apomorphine and oral mercuric chloride. They concluded that the ala cinerea was the VC mediating the vomiting response to all emetic stimuli.

Koppanyi [1930] observed that ala cinerea lesions in chronic dog preparations reduced emetic sensitivity to intravenous

apomorphine but not to orally administered irritant emetics. He, therefore, questioned the concept of the ala cinerea as the VC. *Borison* [1948] and *Borison and Wang* [1949] elicited vomiting in decerebrate cats with electrical stimulation of the reticular formation near the nucleus of the tractus solitarius (NTS). No adjacent sites were sensitive, and the vomiting ceased with cessation of the electrical stimulus.

In chronic dog preparations, these workers observed that superficial medullary lesions with minimal damage to the ala cinerea abolished emesis to intravenous apomorphine and certain cardiac glycosides but not to oral copper sulfate [*Wang and Borison*, 1950, 1951a]. Deeper lesions involving the lateral reticular formation impaired the response to copper sulfate as well as to intravenous apomorphine. Destruction of this portion of the reticular formation by implanted radon seeds in chronic dogs elevated the emetic threshold to apomorphine, digitalis glycosides and copper sulfate [*Borison and Wang*, 1951; *Wang and Borison*, 1951b]. As a result of such observations these workers formulated the concept that a chemoceptive trigger zone for emesis (CTZ), mediating the vomiting response to certain classes of emetic drugs, is located in the ala cinerea, and the VC, controlling the emetic response to all emetic stimuli, is located in the dorsal portion of the reticular formation in the lower medulla oblongata. In another study in 1951 employing selective lesions techniques, *Borison and Brizzee* concluded that the CTZ in the cat is located in the area postrema (AP) rather than the ala cinerea.

Subsequently it has been reported that the AP in several mammalian species appears to mediate the emetic response to digitalis compounds [*Borison and Brizzee*, 1951], ergot alkoloids [*Wang and Glaviano*, 1954], tartar emetic [*Borison and Wang*, 1953], opiate derivatives [*Borison*, 1958], the early emetic response to ionizing radiation [*Brizzee*, 1956] and motion sickness in dogs [*Wang and Chinn*, 1954].

In our more recent investigations we have focussed on the squirrel monkey of the Bolivian strain which we believe may offer certain advantages over the dog and cat as a model for motion sickness studies. Preliminary tests of the sensitivity of this animal model to various modalities of motion were carried out recently in our laboratory [*Ordy and Brizzee*, 1980]. In those studies the highest emetic incidence observed was 89% with a mean emetic frequency of 2.0 responses in 60 min, and a mean latency to first response of 19 min at 25 rpm horizontal rotation, and 0.5 Hz linear vertical acceleration with the subject positioned in the axis of rotation. Susceptibility - defined by incidence, frequency, and/or latency of emesis - was significantly higher in Bolivian than in Colombian phenotypes, in the presence of visual cues, and in males. However, susceptibility was not influenced by testing at different periods of the day.

The effects of repeated exposures to the motion regimen have also been studied recently in another series of tests [*Brizzee*, unpubl. results, 1982] in the same species. In these studies, which involved successive daily 1-hour test exposures to the standard motion regimen, three basic patterns of response were observed. In one pattern the animals habituated in about 5 days and were refractory for the last few days of the test series. After a rest period of 1 month

the animals responded with essentially the same pattern as in the first tests. In a second pattern the animals habituated very quickly - after only two daily 1-hour tests and responded in a similar fashion when tested by the same regimen after a rest of 1 month. In a third pattern the animals failed to habituate in that they never became refractory to motion-induced emesis over a period of 15 days. However, they did exhibit a decrease in the number of responses during the 1-hour test period from about 5 responses on the initial day of the series to about 2 responses on the 15th day. After a rest period of 1 month the animals exhibited essentially the same pattern of response on a second 15-day test series as they exhibited on the first series.

Following these tests on the sensitivity of Bolivian squirrel monkeys to rotary and vertical motion we performed a series of lesion experiments in which the animals were motion tested at 3-weekly intervals before and after thermal ablation of the AP or sham ablation of this structure [*Brizzee* et al., 1980]. It was observed that complete ablation of the AP resulted in total refractoriness of such operated animals to the motion regimen. Sham-operated animals were as motion-sensitive postoperatively as they were preoperatively. These results appeared to confirm the results of *Wang and Chinn* [1954] which indicated that the CTZ plays a crucial role in the mediation of the emetic response to motion.

Since the lesion method as employed in the above studies is obviously an invasive technique and can assess the functional significance of only one structure at a time we have undertaken a series of experiments employing the 2-DG method [*Sokoloff*, 1977; *Sharp and Kilduff*, 1981] as modified by *Agranoff* et al. [1980], for the purpose of mapping the structures throughout the pons and medulla oblongata which are activated by the motion regimens which we have previously used to elicit emesis in squirrel monkeys [*Ordy and Brizzee*, 1980; *Brizzee* et al., 1980]. The purpose of the present paper is to present our initial observations from these studies.

Materials and Methods

Six pairs of squirrel monkeys were used in this study. The animals were allowed food and water ad libitum until the morning of the experiment. 24 h before each experiment an indwelling intravenous catheter (Becton-Dickinson, 20G2) was inserted into the femoral vein in each animal employing aseptic surgical procedures under nitrous oxide anesthesia. Following insertion of the cannula, a nylon jacket was fitted to each animal to allow them freedom of movement yet prevent them from disturbing the cannula.

Deoxy-*D*-glucose, 2-(1,2-^{3}H), 37.3 Ci/mmol, and deoxy-*D*-glucose, 2-(1-^{14}C), 51.3 mCi/mmol, in a 9:1 ethanol/water mixture were obtained from New England Nuclear. The 2-DG was evaporated to dryness and resuspended to 0.5 ml sterile saline. In four pairs of animals (^{3}H)-2-DG at a dose of 167μCi/100 g body weight was injected via the femoral cannula in each animal. 2 h after this initial injection these animals were injected with resuspended (^{14}C)-2-DG in 0.5 ml sterile saline at a dose of 16.7 μCi/100 g body weight. In two pairs of animals the dose of (^{3}H)-2-DG was 100 μCi/100 g body weight and the dose of (^{14}C)-2-DG was 25 μCi/100 g body weight. Immediately after the ^{14}C-2-DG injection, 1 animal of each pair was immobilized with Velcro straps on a modification of a nontraumatic restraining device described by *Withrow and Devine* [1959] equipped with a nontraumatic moulded plastic head holder maintaining the head in the sagittal plane. Each animal was then placed in a vertical (upright) position with the head tilted about 20° forward and subjected to a 'standard' motion regimen consisting of horizontal rotary motion at 25 rpm together with a vertical movement of 6 inches at 0.5 Hz in a lighted

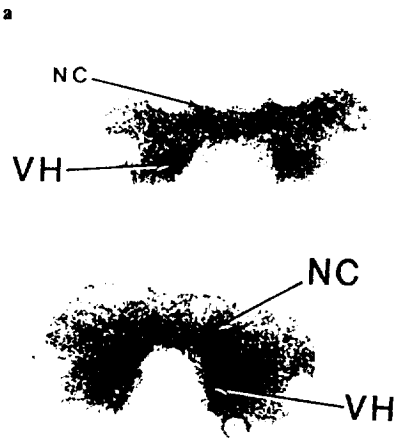

Fig. 1. (^{14}C)-2-DG autoradiographs of transverse cryostat sections of junction zone of spinal cord and medulla. **a** Control, **b** Motion-stimulated. NC = nucleus cuneatus; VH = ventral horn.

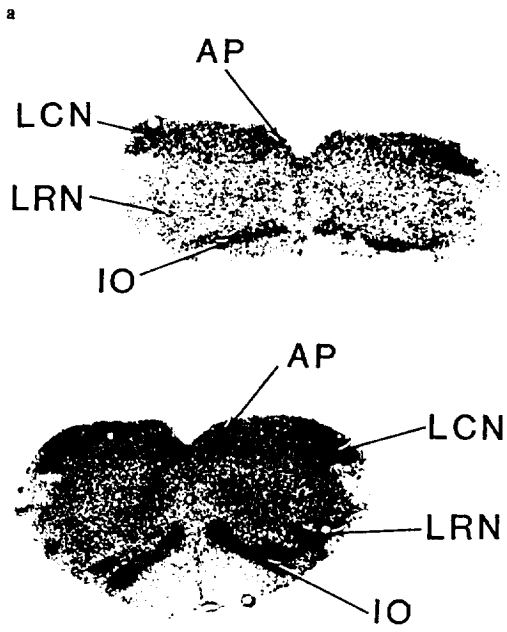

Fig. 2. (^{14}C)-2-DG autoradiographs of transverse cryostat sections of lower medulla. **a** Control. **b** Motion-stimulated. AP = area postrema; LCN = lateral cuneate nucleus; LRN = lateral reticular nucleus; IO = inferior olive.

room for 1 h. The other animal of each pair was restrained in the same manner as the above animal but was maintained in a quiescent state for 1 h.

At the end of this period each animal was anesthetized with ketamine (20 mg/kg) and the brain was quickly dissected out and frozen in isopentane cooled to −60°C with dry ice. Transverse cryostat sections (−15°C) of the brainstem were cut alternately and serially at 200 and 20 μm throughout the medulla and lower pons from the level of the nucleus gracilis through the level of the superior vestibular nucleus. Micropunch samples (brain plugs) were obtained from the 200-μm sections by a modification of the micropunch method of *Eik-Nes and Brizzee* [1965] of the individual vestibular nuclei, the nucleus gracilis, nucleus cuneatus, nucleus of the trigeminospinal tract, nucleus of the solitary tract, hypoglossal nucleus, central reticular formation, dorsal reticular formation and pyramid. The stainless steel punch (trochar) measured 850 μm in diameter. The number of pairs of animals from which 'standard' brain plugs 200 μm deep × 850 μm in diameter were obtained for each brainstem nucleus or structure is shown in table I. In some structures such samples were obtained from only four or five pairs of animals due to technical difficulties.

The punch samples were prepared for liquid scintillation counting by adding distilled water, serum, tissue solubilizer, H_2O_2, glacial acetic acid and aquasol in appropriate amounts and sequence. Liquid scintillation counting was then performed with a Beckman LS7500 system, and differential (^{3}H) and (^{14}C) counts were performed employing external standards.

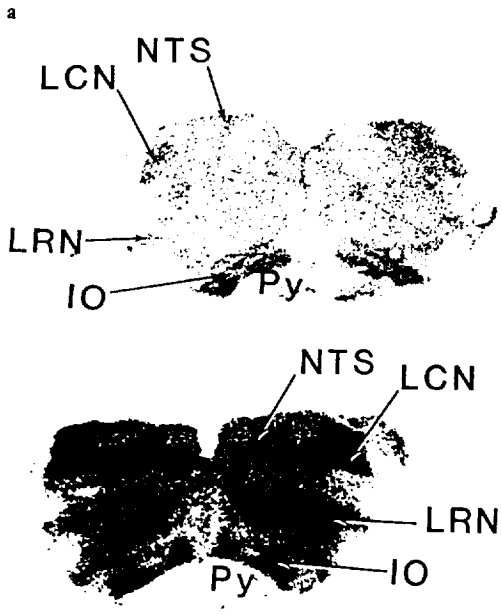

Fig. 3. (^{14}C)-2-DG autoradiographs of transverse cryostat sections of mid-medulla. **a** Control. **b** Motion-stimulated. NTS = nucleus of the tractus solitarius; LCN = lateral cuneate nucleus; LRN = lateral reticular nucleus; IO = inferior olive; Py = pyramid.

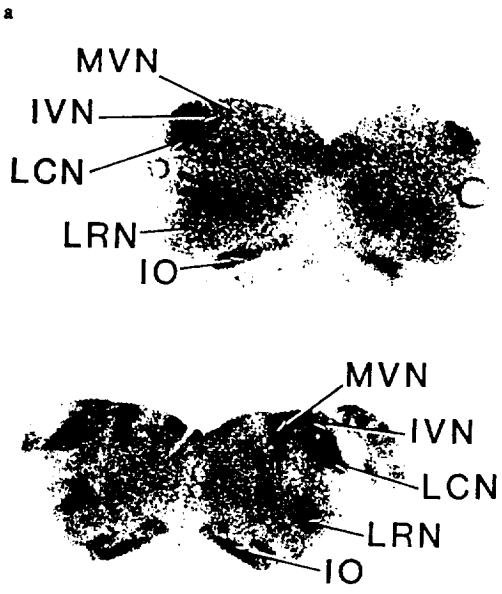

Fig. 4. (^{14}C)-2-DG autoradiographs of transverse cryostat sections of upper medulla. **a** Control. **b** Motion-stimulated. MVN = medial vestibular nucleus; IVN = inferior vestibular nucleus; LCN = lateral cuneate nucleus; LRN = lateral reticular nucleus; IO = inferior olive.

After the frozen micropunch samples were removed the 200-μm sections were placed on micropunch slides, thawed and adhered to the slide by finger warmth applied underneath the slide opposite to the section. They were then fixed in 80% alcohol, stained with toluidine blue and maintained in a slide library as a permanent record of the site of the micropunch samples.

The 20-μm sections were prepared for (^{14}C) autoradiography by standard methods employing Kodak SB-5 X-ray film. Each frozen 20-μm section was placed on a coverslip, dried for 1 min at 60°C on a warming table and stored in a desiccator. The coverslips containing the dried sections were later applied to the SB-5 Kodak X-ray film in a light-tight cassette in the dark. The film was exposed for 10 days and developed in Kodak D-19 developer.

Results

The autoradiographs in various brainstem structures at several rostrocaudal levels are shown in figures 1-6. It will be observed that the intensity of ^{14}C labeling tends to be greater in most of the sections of motion-stimulated brains than in controls. However, this difference appears to be less notable in the gray matter of the upper spinal cord than in the brainstem (fig. 1a, b). In the lower medulla oblongata the intensity of ^{14}C labeling is particularly high bilaterally in the lateral cuneate nu-

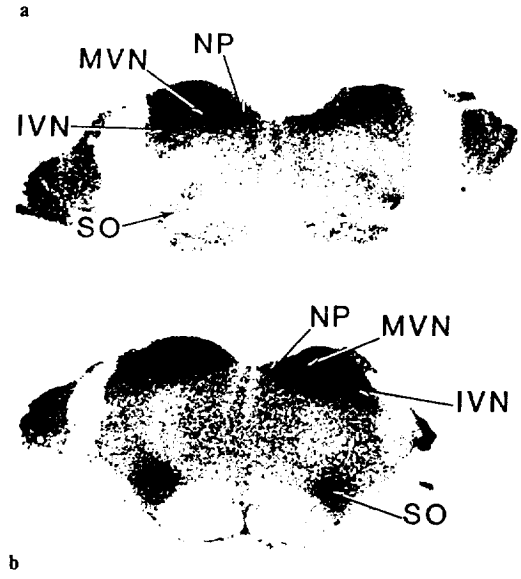

Fig. 5. (^{14}C)-DG autoradiographs of transverse cryostat sections of junction zone of medulla and pons. **a** Control. **b** Motion-stimulated. NP = nucleus prepositus; MVN = medial vestibular nucleus; IVN = inferior vestibular nucleus; SO = superior olive.

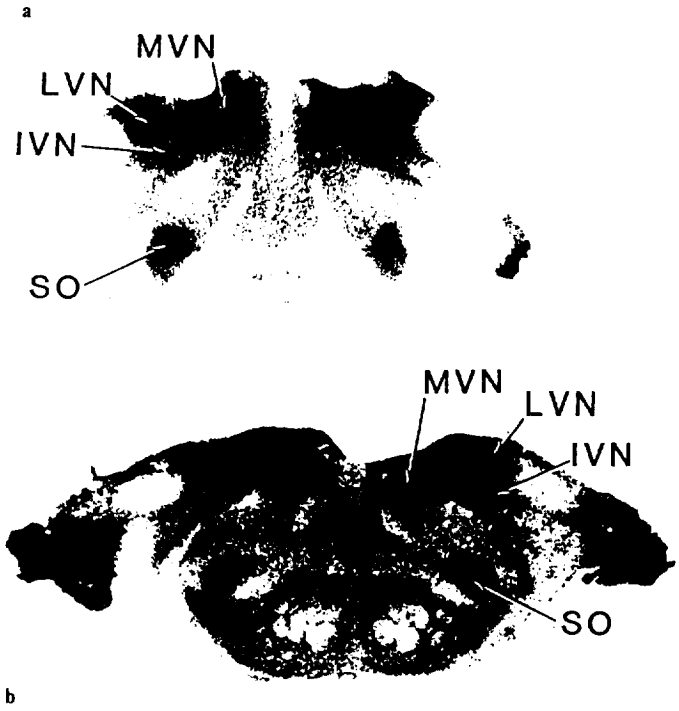

Fig. 6. (^{14}C)-2-DG autoradiographs of transverse cryostat sections of lower pons. **a** Control. **b** Motion-stimulated. MVN = medial vestibular nucleus; LVN = lateral vestibular nucleus; IVN = inferior vestibular nucleus; SO = superior olive.

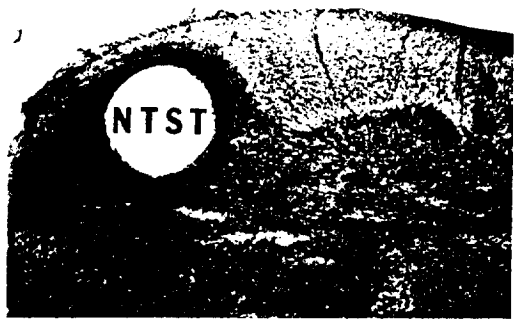

Fig. 7. Transverse cryostat section of junction zone of spinal cord and medulla cut at 200 μm showing site of removal of tissue sample (brain plug) from the nucleus of the trigeminospinal tract (NTST). Toluidine blue.

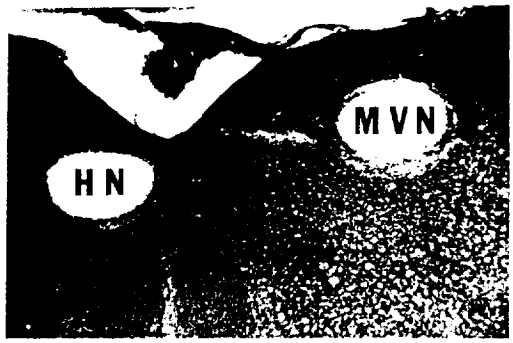

Fig. 9. Transverse cryostat section of mid-medulla cut at 200 μm showing sites of removal of tissue samples (brain plugs) from the hypoglossal nucleus (HN), and medial vestibular nucleus (MVN). Toluidine blue.

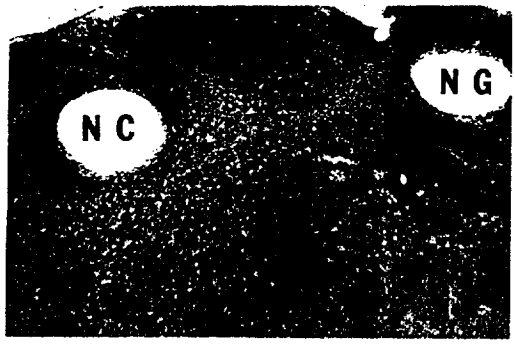

Fig. 8. Transverse cryostat section of lower medulla cut at 200 μm showing sites of removal of tissue samples (brain plugs) from nucleus cuneatus (NC) and nucleus gracilis (NG). Toluidine blue.

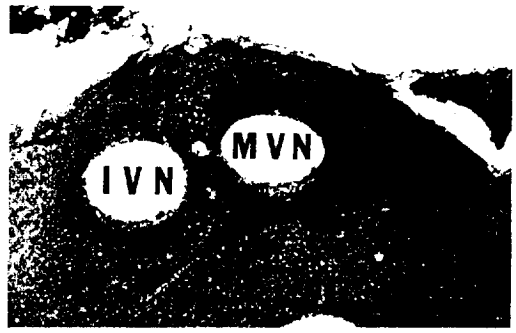

Fig. 10. Transverse cryostat section of upper medulla cut at 200 μm showing sites of removal of tissue samples (brain plugs) from the medial vestibular nucleus (MVN) and inferior vestibular nucleus (IVN).

cleus and inferior olive, and moderately heavy in the AP, in the motion-stimulated as compared with control tissues with a suggestion of increasing labeling in the lateral reticular nucleus (fig. 2a, b). At a slightly more rostral level (fig. 3a, b) the difference between the control and motion-stimulated subjects in the intensity of labeling of the lateral cuneate and lateral reticular nuclei and the inferior olive was also marked. In addition, selectively greater bilateral labeling was also observed in the NTS. At somewhat more rostral levels the labeling of the medial and inferior ves-

Table I. $^{14}C/^{3}H$ 2-DG cpm ratios in motion-stimulated and control animals

Brainstem structure	Number of pairs of animals	% increase in $^{14}C/^{3}H$ 2-DG (cpm) count ratios in motion-stimulated over control animals	
		%	p
Superior vestibular nucleus	4	24,8	NS
Lateral vestibular nucleus	5	24,2	NS
Medial vestibular nucleus	6	21,3	0.0379
Inferior vestibular nucleus	6	27.7	0.0449
Central reticular formation	5	15.0	NS
Dorsal reticular formation (vomiting center)	5	22.9	NS
Hypoglossal nucleus	6	18.8	NS
Nucleus of the solitary tract	4	24.9	NS
Nucleus of trigeminospinal tract	5	20.7	NS
Nucleus gracilis	6	12.1	NS
Nucleus cuneatus	6	23.8	0.0247
Pyramid	6	3.6	NS

tibular nuclei as well as the lateral cuneate nucleus, lateral reticular nucleus and inferior olive was notably more intense in motion-stimulated animals than in controls (fig. 4a, b). In the upper levels of the medulla oblongata (fig. 5a, b) the nucleus prepositus, and medial, lateral and inferior vestibular nuclei were relatively heavily labeled, even in controls. However, the labeling was somewhat more intense in the motion-stimulated subjects.

At the lower pontine levels (fig. 6a, b) the medial, lateral and inferior vestibular nuclei and superior olivary nucleus were consistently heavily labeled in controls (Fig. 6a). As at slightly lower levels in the upper medulla, however, the labeling appeared to be somewhat more intense in the autographs from motion-stimulated subjects (fig. 6b).

Examples of sites of removal of micropunch samples (brain plugs) from brainstem nuclei are shown in figures 7–10. Certain structures in which we observed a marked increase in (^{14}C)-2-DG labeling in the autoradiographs were not punch-sampled since we had not anticipated such an increase in labeling intensity. These included the lateral cuneate nuclei, lateral reticular nuclei and inferior olive. Further, the structural components of the auditory system such as the superior olive and cochlear nuclei which consistently exhibit a heavy 2-DG uptake in controls, were not punch-sampled.

Results of liquid scintillation counts are shown in table I as the percent increase in the $^{14}C/^{3}H$ cpm ratios in these two groups of subjects. The significant increase in the $^{14}C/^{3}H$ cpm ratios of the counts in the medial ($p = 0.0379$) and inferior vestibular ($p = 0.0449$) nuclei and in

the nucleus cuneatus ($p = 0.0247$) suggests that these structures are selectively activated by the motion stimuli. Where the interaction of motion × structure is evaluated, comparing the pyramids and hypoglossal nuclei as 'control' structures on the one hand, and the inferior and medial vestibular nuclei as target structures on the other, the difference between the two for $^{14}C/^{3}H$ cpm is highly significant ($p = 0.0057$). Some of the other structures listed in table I also exhibited relatively high percentage increase in the $^{14}C/^{3}H$ cpm ratio, but the values did not attain the level of statistical significance. This may have occurred due to the fact that some punch samples were lost due to technical difficulties in certain structures. However, since all of them, with the exception of the pyramid, showed higher activity in the motion-stimulated than in the control it seems evident that the motion regimen stimulates widespread increases in glucose metabolism in the medulla oblongata and lower pons.

Discussion

The sequential double-label 2-DG technique appears to offer several potential advantages for studies of brain metabolism. Unlike the autoradiographic 2-DG method, it does not require determination of rate constants for each experimental condition [*Altenau and Agranoff*, 1978]. It does not necessitate monitoring of plasma glucose or DG levels, but does assume that the two injections produce similar plasma curves and that the glucose level is the same under each condition. The sequential double-label method does not yield absolute values of brain glucose metabolism, but rather an accurate ratio of relative rates between brain regions. If a given physiological variable produces a brain asymmetry, ratios can be compared between the affected region and the corresponding contralateral region. The sequential double-label method may be particularly effective, however, in instances where no asymmetry in the radioautographic pattern is produced, in which case the various stimulated brain regions will nevertheless be demonstrable by their higher $^{14}C/^{3}H$ ratios when compared to other, contiguous or remote, unstimulated brain regions. Also, while the microheterogeneity of brain structure is a general problem for regional brain studies, sampling errors are minimized with the sequential double-label procedure, since the first injection provides an internal control for each brain sample. Slight differences in the thickness of the section or the size of brain plugs removed will thus not affect the accuracy of the method.

A statistically significant difference in the $^{14}C/^{3}H$ 2-DG cpm ratio was observed between target nuclei (medial and inferior vestibular) and control structures (hypoglossal nucleus and pyramids) in the quiescent as compared with the stimulated animals. This appears to confirm with quantitative data the observations made in our autoradiographic studies, and the widely accepted concept that these vestibular nuclei are functionally activated by horizontal and vertical motion. The same trend was observed for the lateral and superior nuclei, but the differences did not quite reach the level of statistical significance. Another brainstem nucleus which exhibited significantly higher $^{14}C/^{3}H$ cpm

ratios in activated than in control brains indicative of selectively high levels of (^{14}C)-2-DG uptake was the nucleus cuneatus. This suggests that this nucleus is selectively stimulated by the motion stimuli, presumably due to activation of receptors for proprioception, especially in cervical region of the body.

In the present study the autoradiograph results indicated a selectively heavy uptake in certain structures which might logically be expected to exhibit an enhanced glucose utilization in response to motion stimuli. These include the vestibular nuclei and nucleus prepositus. Other structures which somewhat unexpectedly exhibited an enhanced ^{14}C labeling in motion-stimulated animals were the lateral cuneate nucleus, lateral reticular nucleus and inferior olive. These latter structures also showed a relatively dense ^{14}C labeling in controls, but the labeling appeared to be more intense in the motion-stimulated subjects.

In the micropunch-liquid scintillation counting experiments the number of brainstem nuclei sampled was necessarily less than the number examined autoradiographically. One reason for this is that we sampled only a limited number of nuclei (e.g. vestibular nuclei and nucleus prepositus) which we expected to exhibit an increased glucose uptake upon motion stimulation togehter with certain 'control' nuclei or structures which we did not expect to show such an increase (e.g. hypoglossal nucleus, pyramids). Another reason for not sampling nuclei such as the inferior olive is that the configuration of that nucleus is such that a clean punch sample could not be obtained with the circular 850-μm diameter trochar which we used in this study.

References

Agranoff, B.W.; Boast, C.A.; Frey, K.A.; Altenau, L.L.: Evaluation of regional brain metabolism by a sequential double label 2-deoxyglucose method: in Passonneau, Hawkins, Lust, Welsh, Cerebral metabolism and neural function, pp. 331–337 (Williams & Wilkins, Baltimore 1980).

Altenau, L.L.; Agranoff, B.W.: A sequential double-label 2-deoxyglucose method for measuring regional cerebral metabolism. Brain Res. *153:* 375–381 (1978).

Borison, H.L.: Electrical stimulation of the central nervous mechanism regulating spasmodic respiratory acts in the cat. Am. J. Physiol. *154:* 55–62 (1948).

Borison, H.L.: Influence of area postrema ablation on the emetic effect of adrenaline, apomorphine and pilocarpine administered by cerebral intraventricular injection in the cat. J. Physiol. *143:* 14 (1958).

Borison, H.L.; Brizzee, K.R.: Morphology of the emetic chemoreceptor trigger zone in the cat medulla oblongata. Proc. Soc. exp. Biol. Med. *77:* 38–42 (1951).

Borison, H.L.; Wang, S.C.: Functional localization of central coordinating mechanism for emesis in cat. J. Neurophysiol. *12:* 305–313 (1949).

Borison, H.L.; Wang, S.C.: Quantitative effects of radon implanted in the medulla oblongata: a technique for producing discrete lesions. J. comp. Neurol. *94:* 33–56 (1951).

Borison, H.L.; Wang, S.C.: Physiology and pharmacology of vomiting. Pharmac. Rev. *5:* 193–230 (1953).

Brizzee, K.R.: Effect of localized brain stem lesions and supradiaphragmatic vagotomy on X-irradiation emesis in monkey. Am. J. Physiol. *187:* 567–570 (1956).

Brizzee, K.R.; Ordy, J.M.; Mehler, W.R.: Effect of ablation of area postrema on frequency and latency of motion sickness-induced emesis in the squirrel monkey. Physiol. Behav. *24:* 849–853 (1980).

Eik-Nes, K.; Brizzee, K.R.: Concentration of tritium in brain tissues of dogs given (1,2^{3}H)-cortisol intravenously. Biochim. biophys. Acta *97:* 320–333 (1965).

Hatcher, R.A.; Weiss, S.: The seat of the emetic action of the digitalis bodies. Archs intern. Med. *29:* 690–704 (1922).

Hatcher, R.A.; Weiss, S.: Studies on vomiting. J. Pharmac. exp. Ther. *22:* 139–193 (1923).

Koppanyi, T.: Studies on the pharmacology of the afferent autonomic system. Archs int. Pharmacodyn. Thér. *39:* 275–284 (1930).

Ordy, J.M.; Brizzee, K.R.: Motion sickness in the squirrel monkey. Aviat. Space envir. Med. *51:* 215–223 (1980).

Sharp, F.R.; Kilduff, T.S.: The 2-deoxyglucose neuroanatomical mapping technique. Trends Neurosci. *4:* 144–148 (1981).

Sokoloff, L.: Relation between physiological function and energy metabolism in the central nervous system. J. Neurochem. *29:* 13–26 (1977).

Thumas, L.J.: Über das Brechcentrum und über die Wirkung einiger pharmakologischer Mittel auf dasselbe. Virchows Arch. path. Anat. Physiol. *123:* 44–69 (1891).

Wang, S.C.; Borison, H.L.: The vomiting center. A critical experimental analysis. Archs Neurol. Psychiat. *63:* 928–941 (1950).

Wang, S.C.; Borison, H.L.: A new concept of organization of the central emetic mechanism: recent studies on the sites of action of apomorphine, copper sulfate and cardiac glycosides. Gastroenterology *22:* 1–12 (1951a).

Wang, S.C.; Borison, H.L.: The vomiting center: its destruction by radon implantation in dog medulla oblongata. Am. J. Physiol. *166:* 712 (1951b).

Wang, S.C.; Chinn, H.I.: Experimental motion sickness in dogs: functional importance of chemoceptive emetic trigger zone. Am. J. Physiol. *178:* 111–116 (1954).

Wang, S.C.; Glaviano, V.V.: Locus of emetic action of morphine and hydergine in dogs. J. Pharmac. exp. Ther. *111:* 329–334 (1954).

Withrow, G.; Devine, M.C.: A new restraining device for testing antiradiation drugs in monkeys. Lab. Anim. Sci. *22:* 419–421 (1959).

K.R. Brizzee,
Tulane University,
Delta Regional Primate Research Center,
Covington, LA 70433 (USA)